EXHIBIT A

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Issam RAAD, Hend A. HANNA, and Nabeel NABULSI	Group Art Unit: 1744
Serial No.: 10/044,842	Examiner: Chin, Brad Y.
Filed: January 11, 2002	Atty. Dkt. No.: UTSC:669US
For: NOVEL ANTISEPTIC DERIVATIVES WITH BROAD SPECTRUM ANTIMICROBIAL ACTIVITY FOR THE IMPREGNATION OF SURFACES	

DECLARATION OF ISSAM RAAD, HEND HANNA, AND NABEEL NABULSI UNDER 37 C.F.R. § 1.131.

We, Issam Raad, Hend A. Hanna, and Nabeel Nabulsi, hereby declare as follows:

1. We are the named inventors for the above-referenced patent application.

2. Prior to September 25, 1998, we conceived of the idea of the idea of preparing compositions that include a basic reagent and a dye, and methods for disinfecting or sterilizing a surface that involve applying to the surface a composition that includes a basic reagent and a dye.

3. As evidence of conception of the invention, attached as Exhibits 1-6 are copies a literature searches we conducted to assess what was known in the literature pertaining to certain anti-infective agents, two of which chlorhexidine and berberine. Our idea was to combine these

agents in a single composition, and to coat the surface of medical devices (such as central venous catheters) with these compositions in an effort to inhibit the growth of microbacterial organisms that cause device-related infections. The date of this search was prior to September 25, 1998. Berberine, one of the agents that we searched in our review of the literature, is a yellow plant dye. Chlorhexidine, another of the agents search in the literature review, is a basic reagent.

4. Furthermore, from prior September 25, 1998 until we filed our provisional application on January 12, 2001, we were diligent in conducting studies to prepare compositions of our invention and evaluate their effectiveness as antimicrobial compositions. During this period, there was continual activity on our lab on this project. As evidence of this activity, we provide Exhibits 7-16, which include additional literature searches for basic reagents and dyes (7-15), and a summary of experiments performed after September 25, 1998, but prior to January 12, 2001, which showed the efficacy of combining various basic reagents and dye (16). In this regard, references to "Gendine" are to a combination of Gentian violet (also noted at "Gv") and chlorhexidene), and references to "PCMX" are to **[INSERT]**.

5. We hereby declare that all statements made by our own knowledge are true and all statements made on information and belief are believed to be true and further that statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment under § 100 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issued thereon.

Date 11/14/05

Issam Raad

Date _____

Hend A. Hanna

Date _____

Nabeel Nabulsi

Genidine [Gentian violet (GV) · Chlorhexidine(CHX)]

Impregnating with DCM solution of Genidine.

	$MRSA_{2066}$	Ps_{4205}	C. Parap.$_{1\text{-}100\text{-}0022}$
PVC[1] (7.0 mm I.D)	28:28[24:26]	21:21[14:15][1]	27:28[25:25]
Si[1]	17:17 (18:19)[1][21:20]	5:3 (11:12)[1][6:0]	21:21(19:19)[1][23:24]
PU[1] (2Lumen; 10 FR.)	21:21[19:19]	14:13*[14:14]	27:26[21:21]
Suture[1](silk)	17:17[16:16]	5:3[2:3]	21:21[12:12]

[1]Immersed for 10 min.
[1]Immersed for 2 h. except for the 10 FR. Silicone, which were immersed for 20 h.
[]Values are for 5 FR. Single lumen and those in parenthesis are for a double-lumen Cook 10.0 FR catheter.
*Gave a 17 mm zone against the multi-resistant PS4277, while minorifampin control yielded 3 mm.
[]values between [ ] are for addition of 2 eq. Base instead of 3 eq.

Second Trial:

	$MRSA_{2066}$	Ps_{4205}	C. Parap.$_{1\text{-}100\text{-}0022}$
PVC[1](7.0 mm I.D)	28:29	22:23	27:27
Si[1] (2 lumen,10 FR)	19:19 (19:20)$^{\phi}$	10:11 (12:13)$^{\phi}$	18:18 (24:25)$^{\phi}$
PU[1] (2lumen; 10 FR.)	22:22	15:15	22:23
Suture[1]	15:15	4:4	14:14

[1]Immersed for 1h.
[1]Immersed for 2h.
$^{\phi}$Values in parenthesis are for 20h immersion.

Control 1[1]: CHX in (DCM + Methanol)[1] and in Methanol

	$MRSA_{2066}$		Ps_{4205}		C. Parap.$_{1\text{-}100\text{-}0022}$	
	DCM+MeOH	MeOH	DCM+MeOH	MeOH	DCM+MeOH	MeOH
PVC	0:0	0:0	0:0	0:0	0:0	0:0
Si	0:0	0:0	0:0	0:0	0:0	0:0
PU	17:17	11:11	10:10	0:0	15:15	0:0
Suture	0:0	0:0	0:0	0:0	0:0	0:0

[1]All immersed for 2 h.
[1] About 33% DCM/MeOH (v/v)

Control 2: GV in DCM and methanol

	$MRSA_{2066}$	Ps_{4205}	C. Parap.$_{1\text{-}100\text{-}}$

					0022	
	DCM	MeOH[ϕ]	DCM	MeOH[ϕ]	DCM	MeOH[ϕ]
PVC[▯]	25:25	20:21	0:0	0:0	27:27	18:19
Si[▯] (2 lumen,10 FR)	6:7	7:8	0:0	0:0	0:0	0:0
PU[▯] (2lumen; 10 FR.)	22:22	32:32	0:0	0:0	22:23	31:32
Suture[▯]	8:8	10:11	0:0	0:0	0:0	0:0

[▯]Immersed for 10 min. [▯]Immersed for 2 h.
[ϕ]All devices immersed for 2 h.

Genidine in methanol:

	$MRSA_{2066}$	Ps_{4205}	C. Parap.-1-100-0022
PVC	24:25	13:13	23:23
Si	10:12	0:0	0:0
PU	17:17	7:0	16:17
Suture	10:10	0:0	5:6

Experimental:

A. Impregantion Procedure

7.35 ml of 1M solution potassium *t*-butoxide in THF was added to a solution of CHX diacetate (1.533g; 2.45 mmol) in 35 ml THF. The resulting heterogeneous solution was stirred for 20 min, then added to a solution GV (1.0 g; 2.45 mmol) in 30 ml THF. The mixture was stirred at ambient conditions for 1 h, then placed under the hood overnight to evaporate the solvent. The resulting residue was dissolved in 30 ml DCM. One-centimeter device segments were immersed in the DCM solution for the appropriate periods: PVC & PU for 10 min; Si & Silk Suture for 2 h. After removal of the devices from the solution, traces of solution was removed from the lumen, then placed under the hood to dry over night. The impregnated devices were washed with distilled water until the washings were colorless or very faint, then placed under an aseptic hood to dry under ambient conditions for at least 4 h, preferably over night.

B. Zones of Inhibition

BBL Mueller Hinton II agar plates were inoculated with 0.5 McFarland of the appropriate microorganism. The impregnated devices were embedded in the inoculated plates and placed in an incubator at about 37.5° for at least 18 h. Zones of inhibition were then measured and corrected for yeast after incubation for several additional hours.

Durability of Polyurethane & Silicone Impregnated with GV·CHX

Zones of Inhibition against $MRSA_{2066}$.

Catheter	*Day=0*	*Day=3*	*Day=7*	*Day=10*	*Day=17*	*Day=30*	*Day=45*	*Day=61*
PU	21:21	18:18	16:16	15:14	14:14	11:11	8:8	
Si	21:21	18:18	15:15	11:11	11:11	7:8	0:0	
	Day=75							
PU								
Si								

Alternative preparation of Gendine

The neutral form of chlorhexidine [55-56-1] is used instead of the salt form. Hence, .0025 mol of chlorhexidine is added to a stirring heterogeneous solution of 1 g of GV in 60 ml anhydrous THF at room temperature, and the resutling mixture is stirred for 1 h, then placed under the hood to evaporate the solvent. The resulting residue was dissolved in 30 ml DCM. The product did not totally dissolve in DCM.

	$MRSA_{2066}$		Ps_{4205}		C. Parap.1-100-0022	
	Salt	Neutral	Salt	Neutral	Salt	Neutral
PVC	28:28	29:29	21:21	19:19	27:28	30:30
Si	18:19	21:21	11:12	9:9	21:21	22:22
PU	21:21	23:23	14:13	12:12	27:26	22:22
Sutu re	17:17	16:16	5:3	2:4	21:21	15:15

Genidine in methanol:

Again placed under the hood to evaporate DCM. The resulting residue was dissolved in 30 ml MeOH, but the residue did not totally dissolve.

	$MRSA_{2066}$		Ps_{4205}		C. Parap.$_{1\text{-}100\text{-}0022}$	
	Salt	Neutral	Salt	Neutral	Salt	Neutral
PVC	24:25	21:22	13:13	12:12	23:23	20:20
Si	10:12	15:15	0:0	0:0	0:0	8:9
PU	17:17	19:19	7:0	12:12	16:17	12:12
Suture	10:10	12:13	0:0	0:0	5:6	7:7

Durability of Gendine-Coated Silicone UT Catheter

Zones of Inhibition against $EN_{3836(VRE}$ and E. Coli $_{3226}$ after immersion in Urine.

Organism	*Day=0*	*Day=3*	*Day=7*	*Day=14*	*Day=21*	*Day=28*	*Day=35*	*Day=42*	*Day=49*
EN_{3836}	23:23	20	19:19	17:15	13:15	14:13	13		
E. Coli	18:18	15	13:13	13:12	12:12	12:11	11		

Day=0 against $MRSA_{2066}$ = 26:27; against Ps = 18:18; against C. parap. = 24:25

Durability of Gendine-Coated ET PVC Tube

Zones of Inhibition against PS after immersion in Urine.

Organism	*Day=0*	*Day=3*	*Day=7*	*Day=14*	*Day=21*	*Day=28*	*Day=35*	*Day=42*	*Day=49*
EN_{3836}	23:23	20	19:19	17:15	13:15	14:13	13		

Impregnating with Gendine in BuOAc at 40° C

The procedure is that adopted from the patent. CHX was added to a solution of GV in 30 ml *n*-BuOAc at room temp, then about 3 ml of MeOH was added. The resulting mixture along with the devices were heated for one hour at 40°C.

	$MRSA_{2066}$	Ps_{4205}	C. Parap.$_{1\text{-}100\text{-}0022}$
PVC	27:27	17:17	26:26

Si	17:17	0:0	18:18
PU	19:19	13:14	20:20
Silk	14:14	5:5	14:14

Impregnating with Gendine in 25 ml DCM + 5 ml BzOH

	$MRSA_{2066}$	Ps_{4205}	C. Parap.$_{1\text{-}100\text{-}0022}$
PVC	23:23	13:17	23:23
Si	18:18	0:0	17:17
PU	20:20	17:17	20:20
Silk	11:11	0:0	9:9

Impregnating Biliary Stents with Gendine

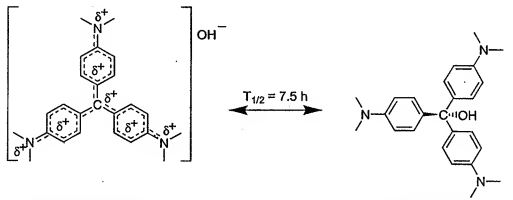
One-centimeter segments were immersed in a DCM solution of Gendine over night. After drying overnight at ambient conditions, the segments were placed in a tube, washed with distilled water, then dried under the aseptic hood over night at ambient conditions. Then the impregnated pieces were embedded in inoculated Mueller-Hinton agar plates, and incubated over night. The resulting zones are given below.

Zone of Inhibition (mm) for Gendine-impregnated Biliary Stents.

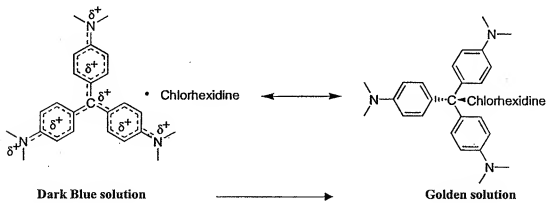
Microorganism	Zone
$MRSA_{2066}$	20
Ps_{4205}	8
E. $Coli_{3202}$	10:11
E. $Coli_{3203}$	11:11
E. $Coli_{3226}$	11
Kb_{2461}	9:9
Kb_{2548}	9:10
Kb_{2556}	6:10
EN_{3836}(VRE)	17
C. $Albican_{64551}$	25
C. $Parap_{1-100-0022}$	18

I. Characterization and Structure of Gendine

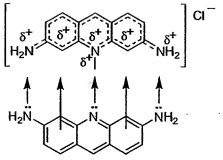
The molecular structures and electronic states of gentian violet has been the subject of extensive studies driven by the observed inhomogeneity of its absorption spectra.[1] In agreement with our observations, Goldacre and Phillips demonstrated the bleaching of gentian violet in the presence of hydroxide. They attributed bleaching to nucleophilic attack of the hydroxide unto the benzylic carbonium center.[2]



Similar bleaching is also observed in our laboratory for Gendine, both in methanol and dichloromethane. This is consistent with the presence of both uncharged and a charged gentian violet moiety in Gendine, where the latter is responsible for imparting the color

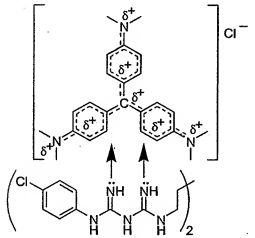


due to the extensive electronic delocalization. Consequently, and similar to that found for the acridinium dye Acriflavin[3], Gendine must exists initially as an EDA (electron Donor-

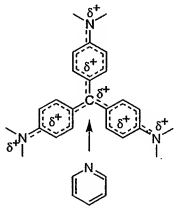


Acrilflavin

Acceptor) complex formed between the cationic gentian violet and chlorhexidine, which is also similar to that observed for gentian violet and pyridine (GV^{+}·Py).[4]

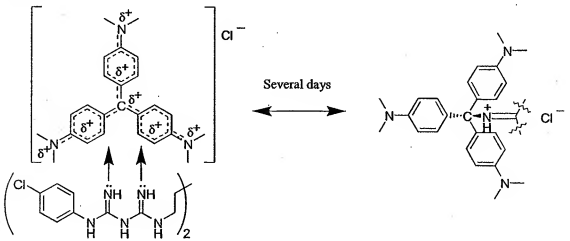


Gendine



GV^{+}·Py

As a result, and at any given time, a solution of Gendine can consist of a mixture of both structural isomers. Meanwhile, zones of inhibition exhibited by devices impregnated with



Dark blue solution

Golden Solution

both solutions rule out conformational isomerism and confirm the presence of structural isomers, since it is expected for conformational isomers to impart similar zones. The data given below support existence of the two structural isomers for Gendine, the EDA "charge-transfer" complex and the covalently bonded isomer.

Zones of Inhibition (mm) for Gendine-Impregnated Devices

	Blue Solution			Golden Solution		
	MRSA	PS	C. Parap.	MRSA	PS	C. Parap.
PVC[1]	28:28	22:22	27:28	22:23	15:15	21:21
PU[1]	21:21	15:15	22:23	19:19	13:13	17:17

[1]The color of the device is dark blue when impregnated with the blue solution, and is light gold (turns darker over night) when impregnated with the light golden solution.
[1]The color of the device is dark blue when impregnated with the blue solution, and is light gray when impregnated with the light golden solution.

II. Durability and Stability

For long-term intravenous therapy, Schierholz *et al.* pointed out the importance of continued release (exceeding 10 days) of antimicrobial, and attributed failure of infection prevention with chlorhexidine- silver sulfadiazine coated catheters to the decreased release beyond 48 hours.[5]

For gendine-impregnated catheters, the efficacy and stability in human serum is being studied for polyurethane (PU) and silicone (Si). As of this date, results show continued release of gendine beyond 30 days (Table 1).

Table 1. Zone of Inhibition (mm) against $MRSA_{2066}$ after incubation in Human Serum .

Catheter	*Day=0*	*Day=3*	*Day=7*	*Day=10*	*Day=17*	*Day=31*	*Day=45*	*Day=60*
PU	21:21	18:18	16:16	15:14	14:14	11:11		
Si	21:21	18:18	15:15	11:11	11:11	7:8		

References

[1] Maruyama, Y.; Ishikawa, M.; Satozono, H. *J. Am. Chem. Soc.* **1996**, *118*, 6257-6263.
[2] Goldacre, R. J.; Philips, J. N. *J. Chem. Soc.*, **1949**, 1724-32.
[3] *Merck Index* **12**, 125.
[4] Liang, E. J.; Ye, X. L.; Kiefer, W. *J. Phys. Chem..*, **1997**, *101*, 7330-7335.
[5] Schierholz, J.; Lefering, R.; Neugebauer, E.; Beuth, J., König, D-P. Pulverer, G. Central Venous Catheters and Bloodstream Infection, *JAMA*, **2000**, *28*, 477.

Comparing zones of inhibition against *pseudomonas aeruginosa* that was cultured from Ruth A. Morrison (# 421222) as imparted by (a) the cook triple lumen polyurethane cvc, and (b) Gendine-impregnated triple lumen polyurethane.

Origin	Cook	Gendine
Peripheral	4 mm	13 mm
CVC	4mm	13mm

Brilliant Green

BG in DCM & MeOH

	$MRSA_{2066}$		PS_{4205}		C. $Parap._{1\text{-}100\text{-}0022}$	
	DCM	MeOH	DCM	MeOH	DCM	MeOH
PVC	30:30	16:17	0:0	0:0	27:27	11:11
Si	0:0	0:5	0:0	0:0	0:0	0:0
PU	20:20	18:18	0:0	0:0	25:25	21:21
Silk	8:9	10:11	0:0	0:0	0:0	7:7

$BG^{+}\cdot I^{-}$ in DCM and Acetone

	$MRSA_{2066}$		PS_{4205}		C. $Parap._{1\text{-}100\text{-}0022}$	
	DCM	Me_2CO	DCM	Me_2CO	DCM	Me_2CO
PVC	18:18	18:19	0:0	0:0	13:13	11:11
Si	16:23	17:18	0:0	0:0	13:13	12:12
PU	20:20	24:24	0:0	0:0	17:17	12:12
Silk	12:12	10:11	0:0	0:0	8:8	7:7

$BG^{+}\cdot CHX^{-}$/DCM

	$MRSA_{2066}$		PS_{4205}		C. $Parap._{1\text{-}100\text{-}0022}$	
	DCM[1]	MeOH[1]	DCM	MeOH	DCM	MeOH
PVC	23:23	21:21	18:18	0:0	18:18	21:22
Si	14:17	15:14	8:9	0:0	18:18	6:8
PU	19:21	16:16	14:14	9:9	17:17	15:15
Silk	11:11	12:13	4:4	2:2	6:7	10:10

[1]slightly soluble. [1]Immersed for 24 h.

Brilliant Green (BG) with & without Chlorhexidine (CHX)*

	$MRSA_{2066}$		PS_{4205}		C. $Parap._{1\text{-}100\text{-}0022}$	
	BG	$BG^{+}\cdot CHX^{-}$	BG	$BG^{+}\cdot CHX^{-}$	BG	$BG^{+}\cdot CHX^{-}$
PVC	30:30	23:23	0:0	18:18	27:27	18:18
Si	0:0	14:17	0:0	8:9	0:0	18:18
PU	20:20	19:21	0:0	14:14	25:25	17:17
Silk	8:9	11:11	0:0	4:4	0:0	6:7

*From DCM

Brilliant Green + Gentian violet, 1:1

	$MRSA_{2066}$		PS_{4205}		C. $Parap._{1\text{-}100\text{-}0022}$	
	DCM	MeOH	DCM	MeOH	DCM	MeOH
PVC	28:28	17:17	~~13:13*~~	0:0	29:29	13:13
Si	8:9	0:0	0:0	0:0	0:0	0:0
PU	24:24	20:20	~~11:12*~~	0:0	26:26	17:17
Silk	7:7	7:7	0:0	0:0	0:0	0:6

*Bacteristatic

Durability of Polyurethane & Silicone Impregnated with GV·CHX

Zones of Inhibition against $MRSA_{2066}$ after x Days Incubation in Human Serum

Catheter	*Day=0*	*Day=3*	*Day=7*	*Day=10*	*Day=17*	*Day=31*	*Day=46*	*Day=61*
PU	21:21	18:18	16:16	15:14	14:14			
Si	21:21	18:18	15:15	11:11	11:11			

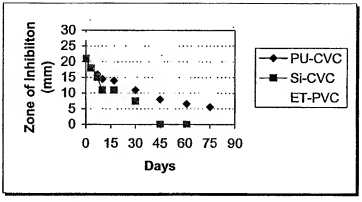
Durability of GV·CHX-coated Silicone UT Catheter

Zones of Inhibition against $EN_{3836(VRE}$ and E. Coli $_{3226}$ after x Days Incubation in Human Urine

Organism	*Day=0*	*Day=3*	*Day=7*	*Day=14*	*Day=21*	*Day=28*	*Day=35*	*Day=42*	*Day=49*
EN_{3836}	23:23	20	19						
E. Coli	18:18	15	13						

Day_0 against $MRSA_{2066}$ = 26:27; against Ps = 18:18; against C. parap. = 24:25

	0	3	7	10	14	17	21	28
PU	21	18	16	**14.5**		14		
Si	21	18	15	11		11		
ET-PVC	28				23		22	22.5

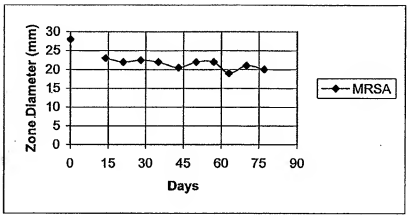


30	**35**	**43**	**45**	**50**	**57**	**61**	**63**	
11			8			6.5		
7.5			0			0		
	22	20.5		22	22		19	.21

75 77
5.5

20

	0	7	10	14	21	28	35	43
MRSA	28			23	22	22.5	22	20.5



50	57	63	70	77
22	22	19	21	20

Durability of Gendine-Impregnated Devices

I. Durability of Polyurethane & Silicone Impregnated with GV·CHX

Zones of Inhibition against $MRSA_{2066}$ after Incubation in Human Serum.

Catheter	*Day=0*	*Day=3*	*Day=7*	*Day=10*	*Day=17*	*Day=30*	*Day=45*	*Day=61*
PU	21:21	18:18	16:16	15:14	14:14	11:11	8:8	6:7
Si	21:21	18:18	15:15	11:11	11:11	7:8	0:0	0:0
	Day=75							
PU	5:6							
Si	ND							

ND = Not done

II. Durability of Gendine-Coated Silicone UT Catheter

Zones of Inhibition against $EN_{3836(VRE)}$ and E. Coli $_{3226}$ after Incubation in Human Urine.

Organism	*Day=0*	*Day=3*	*Day=7*	*Day=14*	*Day=21*	*Day=28*	*Day=35*	*Day=42*	*Day=49*
EN_{3836}	23:23	20	19:19	17:15	13:15	14:13	13:15	15:10	11:17
E. Coli	18:18	15	13:13	13:12	12:12	12:11	11:11	11:11	11:11
	Day=56	*Day=63*	*Day=70*	*Day=78*	*Day=85*	*Day=92*	*Day=99*	*Day=106*	
EN_{3836}	14:17	14:18	14:19	13:16	17:17	17:17	15:15	14	
E. Coli	11:9	0:0	0:0	0:0	-				

Day=0 against $MRSA_{2066}$ = 26:27; against Ps_{4205} = 18:18; against C. $parap._{1\text{-}100\text{-}0022}$ = 24:25

III. Durability of Gendine-Coated ET PVC Tube

Zones of Inhibition after Incubation in Human BAL.

Organism	*Day=0*	*Day=7*	*Day=10*	*Day=14*	*Day=21*	*Day=28*	*Day=35*	*Day=43*	*Day=50*
PS_{4205}	20:20	11:11	0:0						
$MRSA_{2066}$	28:28	ND	ND	23:23	22:22	22:23	22:22	20:21	22:22
	Day=57	*Day=63*	*Day=70*	*Day=77*					
$MRSA_{2066}$	22:22	19:19	21:21	20:20					

ND= Not done

IV. Duarability of Gendine-coated Polyurethane against $MRSA_{2066}$ using methanol as impregnating solvent

Day =0	Day=3	Day=7	Day=10	Day =14	Day=35	Day=46	Day =60	Day=91	
16:16	13:13	ND	12:13	14:14	11:11	10:11	9:9	7:6	

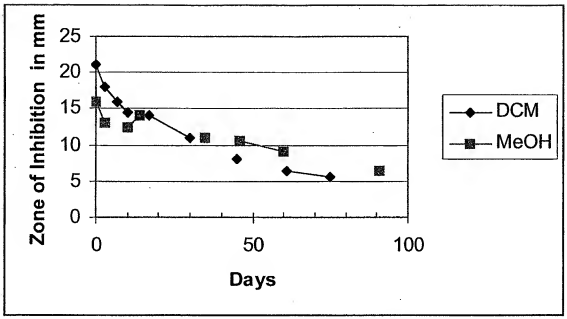


Figure 1. Comparison in durability between DCM and Methanol methods for impregnating polyurethane with Gendine upon incubation at 37° in human serum

Durability of polyurethane impregnated with GV (Gentian Violet).

About 30 one-centimeter sterilized double-lumen catheters (beige) were immersed in a solution of 2 g of GV in 60 ml MeOH (methanol) for 2 h. Catheters were removed from the solution, and traces of solution were removed from the lumen, then allowed to dry over night under the hood. The catheters were washed with distilled water (by shaking the catheters with water in a tube) till the washings were colorless or faint after which the catheters were allowed to dry for at least 4 h. The impregnated catheters were placed in a tube and covered with human serum (Sigma, # S7023), and allowed to stand for the appropriate period @ 37.5° C. Serum was replaced each time catheters were removed. The serum-soaked impregnated catheters were allowed to dry for at least 4 h, then embedded in agar plates (MH II) streaked with appropriate microorganism, and the plates were incubated for 24 h (corrected after 48 h). The resulting zones of inhibition are given below along with the control using cook catheters (impregnated with minocycline-rifampin).

Durability of polyUrethane Impregnated with GV (pUGV).

Zones of Inhibition against $MRSA_{2066}$.

Catheter	*Day=0*	*Day=3*	*Day=7*	*Day=10*	*Day=17*	*Day=31*	*Day=46*	*Day=61*
pUGV	24:24	20:20	19:19	21:21	16:16	15:13	12:12	12:10
Cook*	22:22	17:16	20:20	21:21	29:30	11:11	7:7	0:0

	Day=75
pUGV	9:10
Cook	0:0

*Cook catheter as control

Dr. Raad...

1. Results of doubling the concentration are given in Table 4.
2. Table 5 summarizes results for GV^{+} PCMX using DCM. Notice the zones for silicone.
3. The SIC catheters from Jim Yardly arrived yesterday. I have not done the experiments. I am trying to get hold of Al because I need the voltmeter.
4. Obtained similar zone of inhibition against MRSA for the arrow using both commercially available Mueller Hilton and TSA agar plates, where the zone are 18 mm.

Table 4. Comparison Between Zones at Concentrations 1x and 2x.*

	$MRSA_{2066}$		PS_{3681}		C. $Parap_{1-100-0022}$	
Catheter	1x	2x	1x	2x	1x	2x
ET-PVC	24:26	28:29	15:16	20:21	25:29	28:29
SI	12:13	13:13	0:0	0:0	14:15	13:13
PU	20:20	21:21	8:10	12:12	20:21	25:22
Silk	15:15	17:18	0:0	0:0	12:14	18:22

*1x refers to 2.25 mmol of GV^{+}PCMX dissolved in 30 ml MeOH. 2x refers to 4.50 mmol.

Table 5.

	GV^{+} PCMX in DCM		
Catheter	MRSA	Ps	C. Parap.
ET-PVC	25	19	25
SI	20	12	18
PU	19	15	21
Silk	16	0:0	13

*Catheters are dipped in the solution until swelling becomes visible (few minutes).

Best wishes,

Nabeel

Cholestin
polymixin } anti-pseudomonal abx.

Chloramine

Chlorhexidine - thymol varnish (Cervitec

acetic acid
Zinc Chloride

Na hypochlorite

methyl isothiazolone

India "Gentian violet impreg silicone

Dr. Raad

VTI, ventil. assoc.

✓ Comb. Gentian violet
Indian Medical Research
get article

works less: ethane, methane
works best methylene Chloride

x 12/00

* Donna Pfizer 212 733 6632 med fax #

(1) Ichammol (used: glycerol in ear drops)

(6) staph. aureus ZI = 18 mm

Strep. pyogenes ZI = 23 mm

- no activity against proteus mirabilis, Ps. aeruginosa, E. coli (C. albicans weakly inhibited)
- active against G⊕ org.
- has anti-inflamm. action

need anti-candida

G⊖ ✗

(2) Mercurochrome (used in fungal ear infect.

Has antifungal effect: Aspergillus niger

A. flavus

A. fumigatus

Candida

Mucor

(3) ~~0.5% Chlorhexidine gluconate~~ + 0.025% benzalKonium chloride + 4% benzyl alcohol

VS. 10% Povidone iodine → Betadine

- used as solutions for CVC, arter. cath. care
- Chlorhexid. soln. was superior to povidone iodine against G⊕ bacteria
- Chlorhex. soln. was nonsignificantly superior against G⊖ bact. [$p = 0.8$, $p = 0.5$]

④ S-Carboxymethyl cysteine and its monohydrate lysine salt (used orally for otitis media with effusion)

pts. benefited (avoiding surgical R.) 2.31 times more often than similar patients receiving placebo

⑤ Sanguinarine (used as subgingival irrigation for gingivitis

↓ plaque formation and probing depth (index for gingivitis)

⑥ Glycerine - ethanol : G⊕

⑦ ⑧ Sphingosine and Sphinganine (Free Sphingolipids of the stratum corneum)

- 200 micrograms/cm^2 of sphinganine in ethanol (50 microliters of a 1.6% soln.) → up to 3 log reductions in microorg.
- Sphingolipids : antimicrobial agents of the cutaneous barrier

  Strongly inhibit bacteria, fungi (Staph. aureus, C. albicans, Trichophyton mentagrophytes

(8) 0.1%, 0.2% delmopinol (mouth wash)

- sign. reduction in dextran-producing streptococci
- no colonisation by Candida or G⊖ aerob. bact.
- no ↑ in susceptibility to delmopinol

(study for ~~6 weeks~~)

(9) antiseptic solutions (for venous leg ulcers)

- aluminium acetotartrate (Alsol) 1%
- potassium permanganate 0.015%
- acetic acid 0.25%
- Chloramine 0.25%

organisms found in leg ulcers:

- staph. aureus 79%
- G⊖ rods 39%
- S. epid. 21%
- Proteus spp. 21%
- Pseudom. spp. 14%
- No Fungi

* alum. acet., K. perm., Chloramine reduced the # of bacteria (non-signif.)
* Acetic acid reduced S. aureus ($P = 0.002$)
* " " " G⊖ rods ($P = 0.03$)
* Chloramine reduced G⊖ rods ($P = 0.03$)
* Pseud., Proteus, S. epi, Strep. Haemol.^{G} were reduced (non-signif.)

(10) (9) Alkaloids < Berberine (→ may have an anti-inflammatory action through inhibition of DNA synthesis in activated lymphocytes)
palmatine
Sanguinarine

- Inhibit the multiplication of bacteria, fungi and viruses
- Sanguinarine → inhibits Choline acetyl-transferase
- Berberine, palmatine → active at the α-2 receptor
- Berberine & Sanguinine intercalate DNA, inhibit DNA synthesis and reverse transcript
- Sanguinarine affects membrane permeability
- Berberine affects protein biosynthesis

(11) (10) 13-hexylberberine

several 13-alkyl substituted analogs of berberine and palmatine are highly active against Staph. aureus.

- 13-hexyl berberine } 8×, 4× as active as Kanamycine sulfate
- 13-hexyl palmatine }

(12) (11) Povidone-iodine

iodine and iodophors efficacious against meth. resist-Staph. aureus (MRSA), Enterococcus

- no develop. of resistance
- excellent local tolerability of Betaisodona preparations

~~12~~ 13. Povidone-iodine, Na hypochlorite
Killed X. maltophilia, S. marcescens.
* Chlorhexidine 0.2%
didn't Kill X. maltophilia, S. marcescens (after 10 min.)
* Benzalkonium 0.02%
Killed X. maltophilia
(0.1% was needed to Kill S. marcescens)
* Tego-51 (?)
0.02% Killed X. maltophilia

~~13~~ 14. Peroxyacetic acid (local antiseptic)

2/4/00

Initial expriments: PCMX in MeOH + 50% aq. NaOH added to GV in MeOH, stirred for 1 h. Then evaporated solvent. Dissolved residue in DCM

Catheter	MRSA	Ps	C. Parap.
ET-PVC	23:22:25	12:0:19	22:25:25
Si	16:16:20	12:5:12	17:17:18
PU	20:21:19	16:16:15	23:25:21
Silk	12:12:16	0:0:0:0	12:12:13

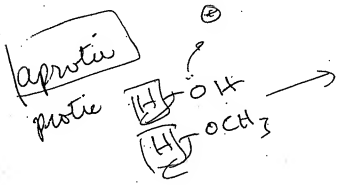
$GV^+ \cdot OH$, prepared by adding 50% aq. NaOH to GV in MeOH, stirring for 1 h, evaporating solvent, dissolving residue in DCM.			
	MRSA	Ps	C. Parap.
PVC	20:20	0:0	17:17
Si	16:16	5:7	14:14
PU	20:20	14:15	25:25
Suture	13:14	0:0	12:12

$GV^+ \cdot OH$, prepared by adding 50% aq. NaOH to GV in water, stirring for 1 h, evaporating solvent, dissolving residue in DCM.			
	MRSA	Ps	C. Parap.
PVC	23:23	0:0	22:22
Si	18:19	9:9	13:16
PU	23:23	17:17	21:21
Suture	17:17	3:4	13:14

$GV^+ \cdot OH \cdot PCMX$, prepared by adding 50% aq. NaOH to GV in water, stirring for 1 h, evaporating solvent, dissolving residue in DCM. Then added PCMX			
	MRSA	Ps	C. Parap.
PVC	22:22	0:0	20:20
Si	21:20	6:10	17:18
PU	25:25	14:14	24:26
Suture	15:16	3:3	13:14

$GV^+ \cdot PCMX$, prepared by adding methanolic sodium methoxide to PCMX in MeOH, then the resulting mixuture is added to GV in MeOH, stirring for 1 h, evaporating the solvent, then dissolving residue in DCM			
	MRSA	Ps	C. Parap.
PVC	21:20	0:0	20:20
Si	18:18	10:10	25:25
PU	21:21	15:15	29:29
Suture	12:13	0:0	13:13

$GV^{+}\cdot OCH_3$, prepared by adding methanolic sodium methoxide to GV in MeOH, stirring for 1 h, evaporating the solvent, then dissolving residue in DCM			
	MRSA	Ps	C. Parap.
PVC	21:20	0:0	20:20
Si	18:19	9:9	25:25
PU	21:21	15:15	28:29
Suture	15:15	0:0	10:13



Comparison between MeOH and DCM solutions of GV.

Catheter	GV in MeOH			GV in DCM		
	MRSA	Ps	C. Parap	MRSA	Ps .	C. Parap
ET-PVC	20-21	0-0	18-19	31-27	18-18	28-31
Si	7-8	0-0	0-0	10-10	0-0	0-7
PU	32-32	24-26	31-32	24-24	18-18	25-25
Silk	10-11	0-0	0-0	9-9	0-0	0-0

1/27/00

Dr. Raad..

This report is to update you on where we are with Gentian violet & PCMX.

I have not received *Clofoctol*. I'll try to recover what we had from the first trials.

Nabeel

1/27/00

Summary of work with $GV^+ \cdot PCMX^-$

Recall that $PCMX^- Na^+$ was added to GV in methanol, and residue resulting from evaporation of methanol was dissolved in DCM. Table 1 shows zones of inhibitions obtained from this first attempt with DCM.

Table 1.

Catheter	$GV^+ \cdot PCMX^-$ in DCM		
	MRSA	Ps	C. Parap.
ET-PVC	25	19	25
Si	20	12	18
PU	19	15	21
Silk	16	0:0	13

dichloromethane
methylene chloride
4-Chloro-~~meta~~-3,5-dimethyl-
cxylenol
vinyl chloride
urethane
suture

The experiment was repeated as follows:
Sodium hydroxide, 0.59 ml of 50% NaOH, was added to 1.15 g (7.35 mmol) of PCMX in 35 ml MeOH. The resulting solution was added dropwise to a solution of GV (3 g; 7.35 mmol) in 150 ml MeOH, and the resulting solution was stirred at ambient conditions for 1 h. The precipitate was filtered under vacuo. The filtrate was placed under the hood over night, allowing the solvent to evaporate. The resulting residue (3.279 g) was used without purification, of which 1.1 g was dissolved in 30 ml DCM for impregnating catheters. Results are given in Table 2.

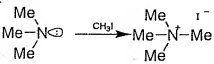
Table 2.

Catheter	$GV^+ \cdot PCMX^-$ in DCM		
	MRSA	Ps	C. Parap.
ET-PVC	22:23	12:0	22:25
Si	16:16	12:5	17:17
PU	20:21	16:16	23:25
Silk	12:12	0:0	12:12

PVC & Si were immersed for 2 min.
PU was immersed for 20 sec.
Silk suture was immersed for 2 h.

do you mind first?

In order to optimize the procedure, it is important to examine the chemistry closely. Careful examination of GV reveals that it is not a true quaternary amine. Quaternary amines have a net positive charge localized on the nitrogen because their lone-pair electrons are involved in covalent bonding with a nucleophilic center. An example is tetramethylammonium iodide (Equation 1).



Eq. 1

Quaternary ammonium compounds (Quats) are used as phase-transfer catalysts (PTC) for solvating organic salts in organic media by increasing the lipophilicity of the organic salt via formation of an ionic compound with the PTC (Figure 1).

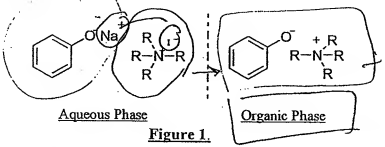
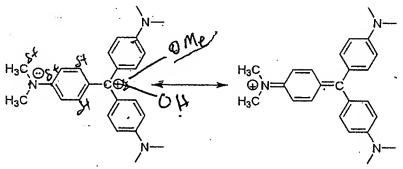


Figure 1.

The case is different for Gentian violet. It is not a true quat. It is an amine with a benzylic carbonium center, which is stabilized by resonance through, in addition to the phenyl rings, delocalizing of the lone-pair of the nitrogens via resonance:



As a result, the positive charge is not localized on the nitrogen. Briefly, and depending on the resonance hybride of GV, it can form ionic compounds with organic salts, and depending on the basicity of the nucleophile, can form covalent adducts at the carbonium center. In other words, the presence of polar protic solvents can compete with the nucleophile.

In the case of PCMX, water, methanol can react with GV at the carbonium center in the presence of a proton acceptor. The phenoxide derivative of PCMX is not a strong nucleophile, but can accept a proton from a hydronium ion. This implies multiple product formation when PCMX is added to a solution of GV in methanol and the presence of water. In other words, the following can result from addition of PCMX to GV in alcoholic aqueous media:

1) $GV^{+} \cdot PCMX^{-}$.
2) $GV^{+} \cdot OH^{-}$.
3) $GV^{+} \cdot OCH_3^{-}$.

The sure way to shed more light on the results on hand, preparing each of these possible reagents and testing their impregnating ability and zones of inhibitions will help shed more light onto the results on hand.

The following results summarize work to date.

Table 3.

GV^+·⁻OH/MeOH			
	MRSA	Ps	C. Parap.
PVC	20:20	0:0	17:17
Si	16:16	5:7	14:14
PU	20:20	14:15	25:25
Suture	13:14	0:0	12:12

Table 4.

GV^+·⁻OCH_3/MeOH			
	MRSA	Ps	C. Parap.
PVC	21:20	0:0	20:20
Si	18:19	9:9	25:25
PU	21:21	15:15	28:29
Suture	15:15	0:0	10:13

Table 5.

GV^+·⁻PCMX/MeOH			
	MRSA	Ps	C. Parap.
PVC	21:22	-0:0	20:20
Si	18:18	10:10	25:25
PU	21:21	15:15	29:29
Suture	12:13	0:0	13:13

Two more experiments in this series are undergoing.

-The experiment employing acetonitrile as a solvent was accidentally spilled.
-I'll set up the rifampin experiment as soon as possible.